

THE WISCONSIN MASTITIS TEST – AN INDIRECT ESTIMATION OF LEUCOCYTES IN MILK¹

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SUMMARY

A simple, rapid screening test for mastitis in bulk samples has been described. The test is based on the observation that an increase in leucocytes is accompanied by an increase in viscosity when a detergent reagent is mixed with a milk sample. The viscosity is determined by measuring the height of a column of milk-reagent mixture remaining in a test tube after a 15-sec outflow through a cap having an orifice 1.2 mm dia. The results of this method correlate well with the square root of the leucocyte count (correlation coefficient equals 0.91).

The problem of the detection and control of abnormal milk has received increased attention in recent years. In 1963 the National Mastitis Council (2) the Public Health Service (4) and the National Conference on Interstate Milk Shipments (3) recommended that states develop mastitis control programs. Recently many municipalities have accelerated their programs for the detection and prevention of abnormal milk.

The screening test generally considered to be the most acceptable yardstick for detecting abnormal milk has been the direct microscopic counting of leucocytes in milk. This test is tedious, time consuming and requires well trained personnel.

The California Mastitis Test (CMT) is a simple and rapid screening test that has gained wide acceptance. Schalm and Noorlander (5) reported that the total cell count in milk is related to the amount of gel formed when the CMT reagent is mixed with milk. A visual judgment is made of the amount of precipitate and thickening as the milk-reagent mixture is rotated in the shallow cups of the white plastic test paddle. Later, Carroll and Schalm (1) concluded that only the nucleated body cells contain the gel forming material.

The Wisconsin Mastitis Test (WMT) described in this paper adapts the principle of the CMT to a

quantitative laboratory screening procedure for herd milk samples. It permits more precise quantitative measurements and is based on the measurement of viscosity as determined by the rate of flow of a mixture of milk and reagent through a standard orifice. The data presented here compares the WMT values with the leucocyte counts (total cell counts).

MATERIALS

(a) The special equipment needed for the WMT consists of plastic test tubes³ measuring 12.5 x 125 mm with a 3-mm (approx.) diameter air vent and with a polypropylene cap⁴ 1 mm thick having a 1.15 mm diameter orifice in its center. The air vent was made 62 mm from the inside bottom of the tubes with a 3-mm diameter nail heated in a bunsen flame (see Figure 1). The holes in the caps were drilled with a 3/64-in. bit in a hand drill. The loose plastic "flaps" and protrusions produced during the drilling of the holes were removed with a razor blade and a piece of a razor blade held with a cover glass forceps having bent spade points. A dissecting microscope was used to facilitate this removal.

(b) The racks used to hold the test tubes were made from single sheets of .040-in. thick aluminum measuring 18 1/2 x 8 in. The finished rack is 3 in. wide; the legs were made by bending down the ends of the bottom support 2 3/8 in. from each end. The distance between top and bottom supports is 2 in. The holes made with a punch and die are 1/2 in. in diameter and are in two rows of 10 holes each, with centers at 1 1/4 in. intervals and 1 1/2 in. between centers of the front and back rows. The rows are 3/4 in. from the hole centers to the edge of the support. Each end of the top support was curled upward in a 1 1/4 in. diameter half circle. A snug fit of the tubes in the holes holds them in place during inversion.

(c) Milk pipette, 2.2 ml (APHA); or 2-ml Corn-

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³Manufactured by Lerner Plastics Inc., Garwood, N. J., described as "flexvials" with nat. "flex-caps".

⁴Suitable metal caps were not commercially available at this writing.

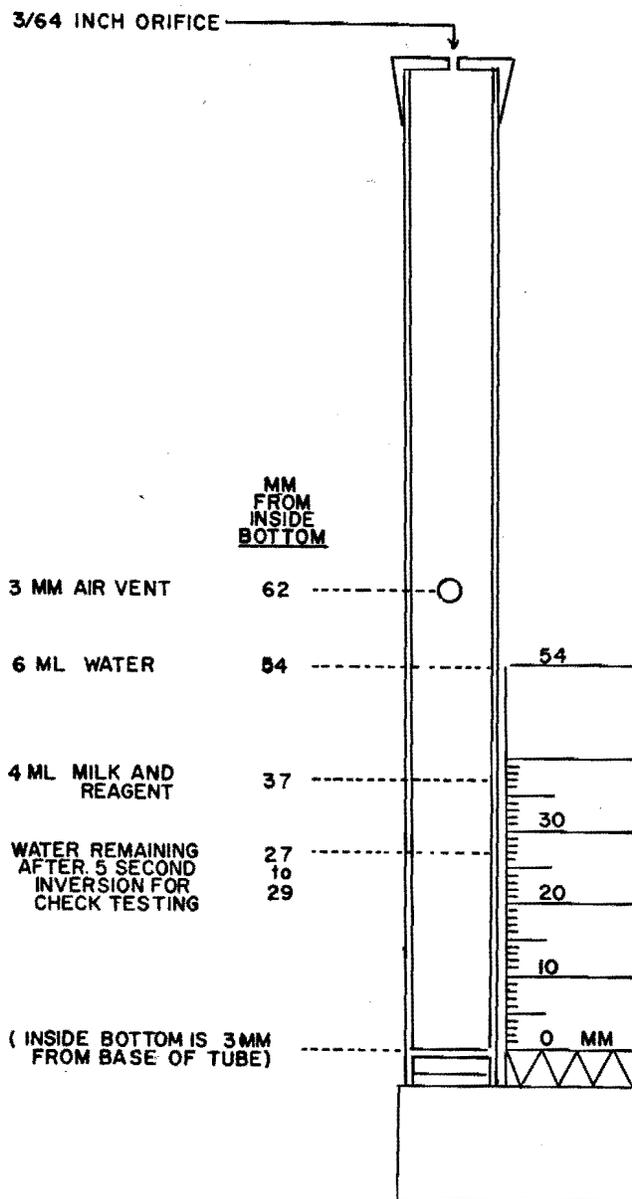


Figure 1. Diagram showing 12.5 x 125 mm test tube and measuring square. Test tube has air vent in side of tube and is equipped with a cap having a 3/64 in. orifice in its center. Measuring square is calibrated in mm. Data for checking accuracy of caps and tubes are shown.

wall syringe and metal pipetting holder, B-D Nos. 1250 S and 1250 MH, equipped with a 2-in. long 14-gauge cannula.

(d) Measuring square made from 1/8-in. thick clear plastic having graduations in mm. The length of vertical arm is exactly 54 mm from the zero line. (See Figure 1).

(e) Cornwall continuous pipetting outfits, 2 ml and 10 ml size, B-D Nos. 1251 and 1271, and one 14-gauge, 4-in. cannula.

(f) Stopwatch or equivalent timing device.

(g) CMT reagent⁵ diluted one plus one with dis-

tilled water. (Detergent content in the diluted reagent was 1.25%, calculated as alkyl benzene sulfonate. The pH was 7.1). Reagent should be standardized since it has been found that supplies from different sources vary.

EXPERIMENTAL PROCEDURES AND METHODS

The accuracy of the caps was checked before each experiment in the following manner: 6 ml of distilled water at 24 ± 2 C was pipetted accurately into the 12.5 x 125-mm plastic test tubes with a 10-ml adjustable Cornwall continuous pipette. The tubes were capped and the rack was inverted rapidly but smoothly, held in a vertical position for 5 sec and returned upright. The caps were considered to be acceptable

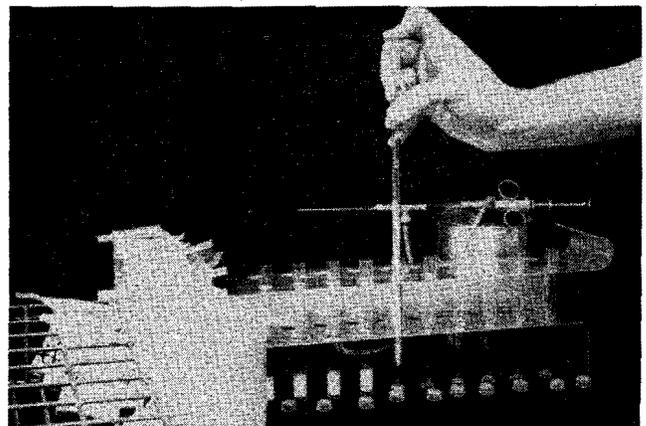


Figure 2 Step 1: Two ml portions of milk sample are pipetted into test tubes.

when the length of water column remaining in the tubes measured 27-29 mm to the top of the meniscus. Check tests for each cap were made in triplicate. This procedure may be used also for developing and maintaining uniformity in technique of inverting and of timing accurately. Jerky movement of rack during inverting or returning upright should be avoided.

The milk samples used were herd samples from farm bulk tanks collected from predominantly Holstein herds during December and January⁶. The samples were kept cold (0-4 C) and WMT values were run on the day of collection. (WMT values decreased as much as 5 to 10 per cent after storage for 24 hours). Three smears were made with a .01-ml pipette (APHA) from each sample on slides having circular areas of 1 cm². The films were stained with Levowitz and Weber stain (6). Forty microscopic fields were counted on each of the 3 milk films using the oil immersion objective. A binocular microscope having a 400,000 factor was used.

The WMT values were determined in the following manner: 2-ml portions of milk samples were

⁵Mfgd. by Norden Laboratories Inc., Lincoln, Nebr. Under U. S. Patent No. 2,998,392. Control No. P 728.

⁶Months of above average milk production.

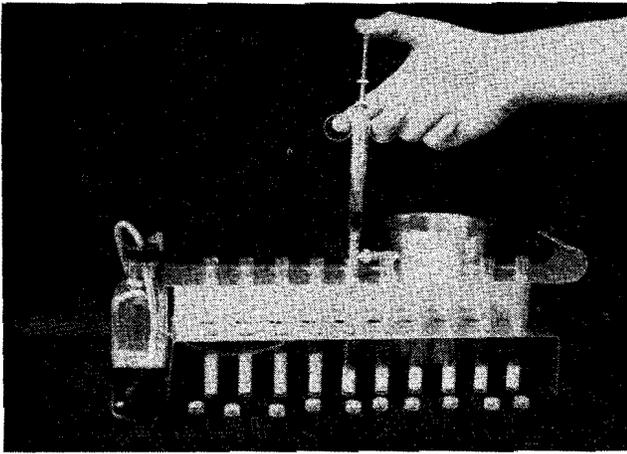


Figure 3 Step 2: Continuous pipetting outfit is used to measure 2 ml of reagent into tubes. Cannula is inserted below the milk surface to prevent foaming.

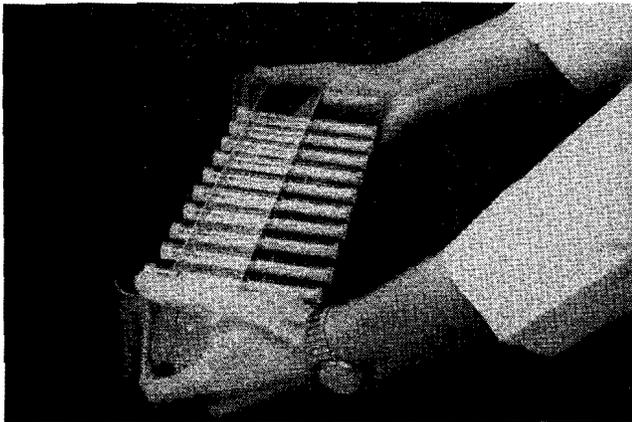


Figure 4 Step 3: The tubes are capped and their contents are mixed by tilting back and forth 10 times in about 10 seconds in a nearly horizontal position. Air vents are placed upward to avoid accidental spillage.

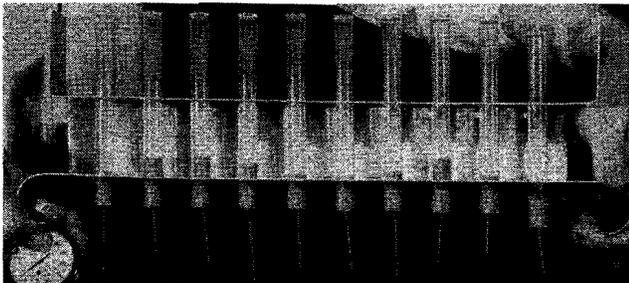


Figure 5 Step 4: Stopwatch is used to time inversion of test tubes for 15 seconds.

pipetted into test tubes (see Figure 2). Then 2 ml of diluted CMT reagent was added to the tubes with a continuous pipetting outfit (see Figure 3). The tubes were then capped with the polypropylene caps with the 3/64 in. orifice. The contents of the tubes were mixed by tilting tubes back and forth 10 times in about 10 sec in a nearly horizontal position (see

Figure 4). Tubes were allowed to stand for 20-30 sec. The tubes were then inverted to a vertical position to permit outflow. The inversion time here was for 15 sec timed with a stopwatch (see Figure 5). The temperature of the milk-reagent mixture at the time of inverting was 24 ± 2 C. Temperature

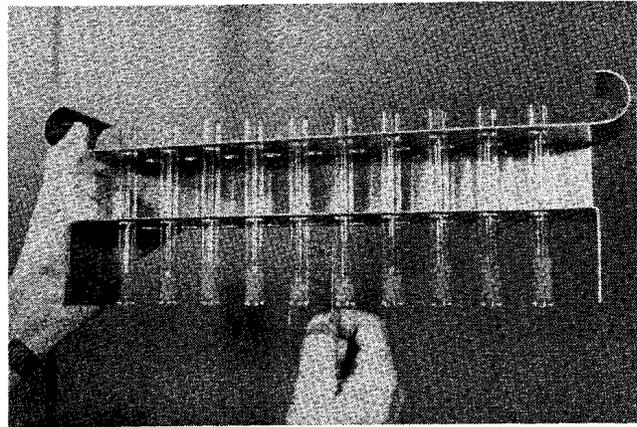


Figure 6 Step 5: Length of liquid columns remaining in tubes is measured in mm with measuring square.

may be adjusted by warming the reagent. The tubes were allowed to stand upright for at least 1 min after the inversion. The WMT values were then recorded in mm as the length of the liquid column remaining in the tube (see Figure 6).

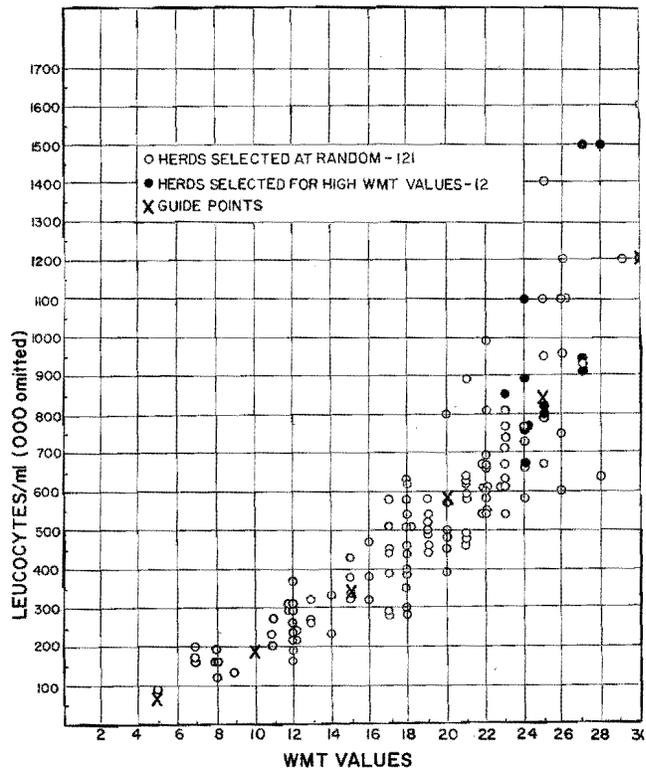


Figure 7. WMT - Leucocyte comparisons.

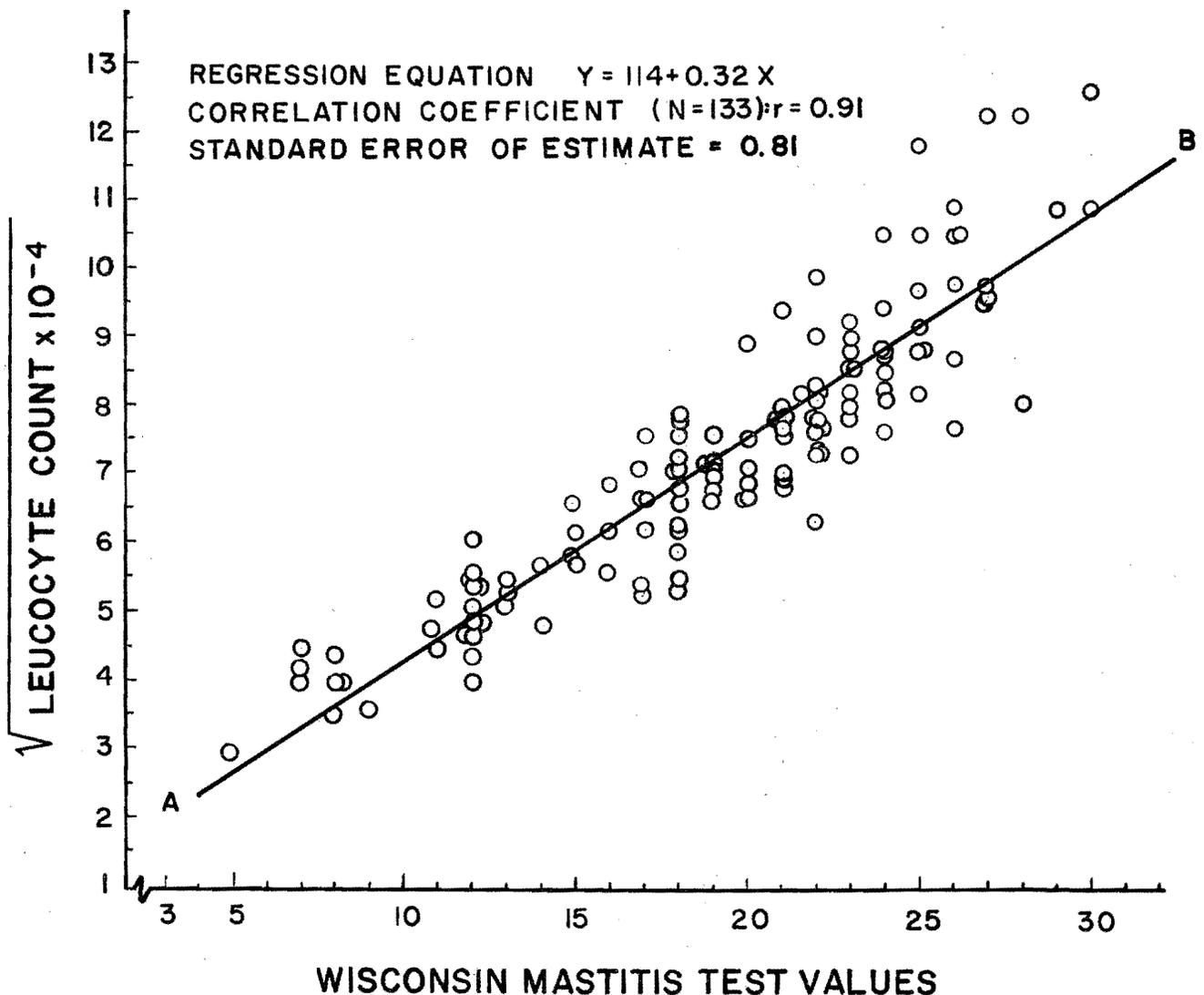


Figure 8. Statistical Data — Square Root of Leucocyte Count vs WMT Values.

For this investigation batches of 10 samples each were run in triplicate using 3 racks with 10 tubes in each rack. It may be advantageous to use all 20 spaces in each rack for routine work with large numbers of samples.

Ten calibrated caps were selected for each day's experiments in a manner previously described. The caps were rinsed immediately after each use by holding upside down in a stream of warm tap water. This was done to remove the slimy deposit that collects around the orifices. The plastic tubes were rinsed 2 or 3 times in warm tap water (not over 45 C) after each use. Hot water may distort tubes. Excess water was removed by shaking the inverted rack before the next use.

RESULTS AND DISCUSSION

The leucocyte count of samples from 133 bulk tanks was determined and compared with the WMT

values. All the determinations were plotted in Figure 7. These results show that the WMT values increase as the leucocyte count increases.

All of the WMT values were also plotted against the square root of the leucocyte count in Figure 8. This graph shows that a more direct relationship exists between the square root of the leucocyte count and the WMT values⁷. This relationship may be used to predict the average leucocyte count from the WMT value. Data from points along the regression line AB were used to locate guide points in Figure 7. The guide points may be used to determine the average leucocyte counts from representative WMT values. For example the average leucocyte count

⁷A possible explanation for the closer relationship between WMT values and the square root of the leucocyte count is: the square root takes into account the hydraulic principle that for liquids of a given viscosity the rate of flow is proportional to the square root of the head (pressure).

for a WMT value of 20 is 570,000 (range 390,000 - 800,000).

Figure 7 presents an easy way to obtain the following information: (a) the number and percentage of herds (those selected at random) that fell above a given WMT value or a given leucocyte count; (b) the range of leucocyte counts obtained for a given WMT value; and (c) the relationship of representative WMT values to the leucocyte count (from the guide points).

A reproducibility study with 30 replicate WMT determinations for each of 3 samples (90 tests) showed the Standard Deviation to be 1 mm. One sample each of low, medium and high leucocyte count was used.

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